Low Molecular Weight Phenolics as Novel Inhibitors of Gastric H+/K+-ATPase

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Abstract-

Low molecular weight phenolics have been identified as promising inhibitors of the H+/K+ATPase of the stomach. This study assessed various phenolics for their inhibitory effect on the ATPase through in vitro enzymatic studies and molecular docking. Various phenolics showed competitive as well as non-competitive inhibition of the enzyme with IC50 ranging in the micromolar concentration, which is similar to the activity levels of conventional drugs. Analysis on the structure-activity relationship showed that hydroxylation and carboxyl group are important for the binding to the ATPase catalytic site. These natural products can be potential leads for antisecretory agents due to their safety and stomach acidity resistance properties.

Introduction-

Excessive secretion of gastric acids leads to conditions such as peptic ulcers, GERD and Zollinger-Ellison syndrome. The H+/K+-ATPase, found in the parietal cells of the stomach, is the terminal component in acid secretion and serves as one of the best targets for pharmacological interventions that inhibit acid secretion. Although proton pump inhibitors like omeprazole prove to be clinically effective, issues surrounding their prolonged usage arise due to malabsorption of nutrients, susceptibility to infections and rebound hypersecretion.

The low-molecular-weight phenolics are common secondary metabolites in plants with reported anti-inflammatory, antioxidant and antimicrobial properties. The small size, acid stability and favorable pharmacology of phenolics are ideal for

their use in drug design. However, studies that evaluate interactions between specific phenolic compounds and membrane ATPases are lacking.

Low molecular weight phenolics have been studied for their role in inhibiting H+/K+-ATPase. Main points that are being studied include:
2. Assessment of different phenolics for their H+/K+-ATPase inhibitory activity by in vitro enzyme assay.
3. Estimation of the inhibition kinetics and mechanism through Lineweaver-Burk plots.
4. Study of structure-activity relationship to find pharmacophore necessary for binding.
5. Study of the interaction through docking studies on the ATPase catalytic subunit.

In addition to efficacy, the reason behind studying phenolic inhibitors includes their safety and environmental sustainability. The current proton pump inhibitors are limited by their pharmacokinetics and potential side-effects in the long term.

Key considerations guiding this work:
2. Structure-Activity Analysis of Low Molecular Weight Phenolics as H+/K+-ATPase Inhibitors: It emphasizes pharmacophore mapping for rational design.
3. Natural Low Molecular Weight Phenolics: A New Class of Gastric Proton Pump Inhibitors* frames the therapeutic novelty and natural product advantage.
4. Inhibition of H+/K+-ATPase by Low Molecular Weight Phenolics: Mechanism and Therapeutic Potential: It highlights mechanistic insight plus clinical relevance.
5. Plant-Derived Low Molecular Weight Phenolics as Selective H+/K+-ATPase Inhibitors: It underscores selectivity to minimize systemic effects.

Materials and Methods-

Chemicals and Reagents-

Phenolic compounds with low molecular weight such as gallic acid, caffeic acid, ferulic acid, p-coumaric acid, vanillic acid, syringic acid, protocatechuic acid and pyrogallol with purity greater than 98% were bought. Lansoprazole was used as a positive control. The other chemicals like ATP, MgCl2, KCl, imidazole, and sucrose were analytical grade. Porcine gastric H+/K+ ATPase was isolated from fresh stomach mucosa procured from a local slaughter house.

Isolation of Gastric H+/K+-ATPase-

The preparation of gastric vesicles with H+/K+-ATPase was performed by

differential centrifugation technique. Scraping of mucosal tissue followed by homogenization in 0.25 M sucrose buffer with 1 mM EDTA and 20 mM Tris-HCl buffer (pH 7.4) followed by centrifugation at 20,000 g for 30 minutes. Further centrifugation was done at 100,000 g for one hour from the supernatant obtained from the previous step. The resultant microsomal pellet was suspended in buffer and then subjected to ultracentrifugation using 7.5% Ficoll gradient at 100,000 g for 90 minutes.

H+/K+-ATPase inhibition test-

The activity of H+/K+-ATPase was determined by measuring K+-stimulated ouabain-insensitive hydrolysis of ATP. The reaction mixture consisted of 50 mM Tris-HCl buffer, pH 7.4; 2 mM $MgCl_2$; 10 mM KCl; 2 mM ATP; 10 µg of the enzyme and 1 mL of reaction medium in total. The inhibitors were solubilized in DMSO and incubated together with the enzyme for 10 min at 37 °C. Final concentration of DMSO in the assay mixture never exceeded 1 %. The reaction was started by adding ATP to the mixture and stopped after 20 min by the addition of cold 12% TCA. Amount of the released phosphate was assayed according to Fiske and Subbarow using absorption reading at 660 nm.

Kinetic studies-

The type of enzyme inhibition was determined using Lineweaver-Burk graphing. The rate of enzyme catalysis reaction was determined with the change in concentrations of ATP from 0.1 mM - 2.0 mM in the presence and absence of selected phenolics at IC50 concentrations.

Molecular Docking-

The crystal structure of H+/K+ -ATPase protein was obtained from the Protein Data Bank. The structure of ligands for phenolic compounds was prepared and energy minimized in ChemDraw 3D. Molecular docking studies were carried out with AutoDock Vina with grid box around ATP binding region. Results for binding affinity and hydrogen bonding/hydrophobic interaction were studied through Discovery Studio Visualizer. The reference molecule used for docking studies was lansoprazole.

Statistical analysis-

Values are presented as the mean ± SD from three separate experiments. The statistical significance of differences between groups was analyzed by one-way ANOVA followed by Tukey's post hoc test using SPSS v26.0. P value < 0.05 was considered statistically significant.

Results and Discussions:

From screening experiments, gallic acid, caffeic acid and protocatechuic acid were found to be strong H+/K+-ATPase inhibitors with IC50 values of 12.4, 18.7 and 21.3 µM, similar to the IC50 value of 8.9 µM for lansoprazole. Kinetics studies revealed that there was a non-competitive inhibitory effect on gallic acid and mixed type inhibition on caffeic acid, which may imply more than one binding mechanism apart from the ATP binding site. Structural activity relationship study revealed that presence of ortho-dihydroxyl and carboxyl functionalities increases inhibitory activity, whereas methoxyl substitution reduces the inhibitory effect. Docking studies proved the presence of stable hydrogen bond formation between Asp386 and Lys791 amino acids in catalytic domain.

Conclusion-

The low molecular weight phenolic compounds like gallic acid and caffeic acid have been proven effective in inhibiting the H+/K+-ATPase enzyme present in the stomach. Owing to their natural source, good safety index and unique modes of action, they have been identified to have potential as future antisecretory drugs.

References-

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